AN IMPROVED METHOD FOR MASS REARING THE TOBACCO HORNWORM

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AN IMPROVED METHOD FOR MASS REARING THE TOBACCO HORNWORM

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ABSTRACT

A semiclosed system that reduced contamination and eliminated handling of individual larvae before they reach the prepupal stage was developed for mass rearing the tobacco hornworm, *Manduca sexta* (L.). To reduce costs and increase vitamin A and its precursors, a diet consisting primarily of corn meal, defatted soy flour, nonfat dried milk, and soy oil (CSM) was substituted for a sucrose, wheat germ, and salt mixture. Larvae on this diet are more normal in color than those reared on the standard wheat germ and casein-yeast diet formerly used. Gelcarin was substituted for agar to reduce cost and simplify mixing of the diet. Artificial leaves treated with a crude tobacco extract eliminated the need for tobacco plants as an oviposition substrate. Rearing losses, egg to adult, were reduced from 85 to 15 percent. Labor to rear insects was reduced from 10.5 man-hours per 1,000 insects to 1.7, or 84 percent, a reduction that allowed 1 man to rear 20,000 insects per week. Sterile hornworm males produced with our techniques were almost 100 percent competitive with native males on St. Croix, U.S. Virgin Islands. Also, the ability to produce large numbers of hornworms provides the potential for mass rearing of various predators and parasites, since hornworm eggs are currently being used to rear the spined stilt bug, *Jalysus spinosus* (Say). The technique works well with the tomato hornworm, *M. quinquemaculata* (Haworth), and shows promise for another sphingid, *Hyles euphorbiae* (L.), being reared to control certain weeds (*Euphorbia* sp.). **KEYWORDS: artificial diets, insect nutrition, insect rearing, *Manduca sexta*, mass rearing *Manduca sexta*, nutrition of *Manduca sexta*.**

INTRODUCTION

During the past 25 years, mass rearing of insects in the laboratory has received increasing attention by entomologists. The advantages of an adequate supply of high-quality insects available at a reasonable cost for sterile releases and for studies of attractants, pathogens, parasites, and predators were thoroughly discussed by Knipling (*14*).[2]

Hoffman and Lawson (*11*) reared the tobacco hornworm, *Manduca sexta* (L.),[3] on tobacco in field cages through the fourth stadium and transferred early fifth-instar larvae

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[2] Italic numbers in parentheses refer to items in "Literature Cited" at the end of this publication.

[3] Lepidoptera: Sphingidae.

to tobacco in the laboratory. Later, Hoffman et al. (*12*) concluded "that a controlled environment must be provided if predictable results are to be obtained." In the absence of laboratory rearing facilities, Allen and Kinard (*1*) met needs for sex pheromone studies by collecting late fifth-instar larvae on tobacco suckers after harvest and transferring them to tobacco plants suspended over pupation bins. Laboratory-reared virgin female tobacco hornworms were used in a major suppression study (completed in 1969) with black-light traps on St. Croix, U.S. Virgin Islands (*5*). However, sterile males released in preliminary studies at Oxford, N.C., in 1967 (unpublished data of senior author) and extensively on Vieques, P.R., during 1968 and 1969 (*4*) had only a minimum effect on native populations. On St. Croix, in 1971 and 1972 (*16*), sterile males reared on an improved diet were highly competitive and reduced the native populations to nondetectable levels until diapausing moths emerged. Several behavioral studies of laboratory and field-reared hornworms were reported by Sparks (*17, 19*). Elsey and Stinner (*8*) used laboratory-produced hornworm eggs to establish a colony of the spined stilt bug, *Jalysus spinosus* (Say), for basic biological studies.

Rearing procedures at Oxford, N.C., evolved from the work of Yamamoto (*20, 21*). Some present techniques at Oxford, including certain aspects of egg production and pupation, are little changed from Yamamoto's methods. Yamamoto developed procedures for rearing 200–500 tobacco hornworms per week under nonsterile conditions in semiopen trays and pro-

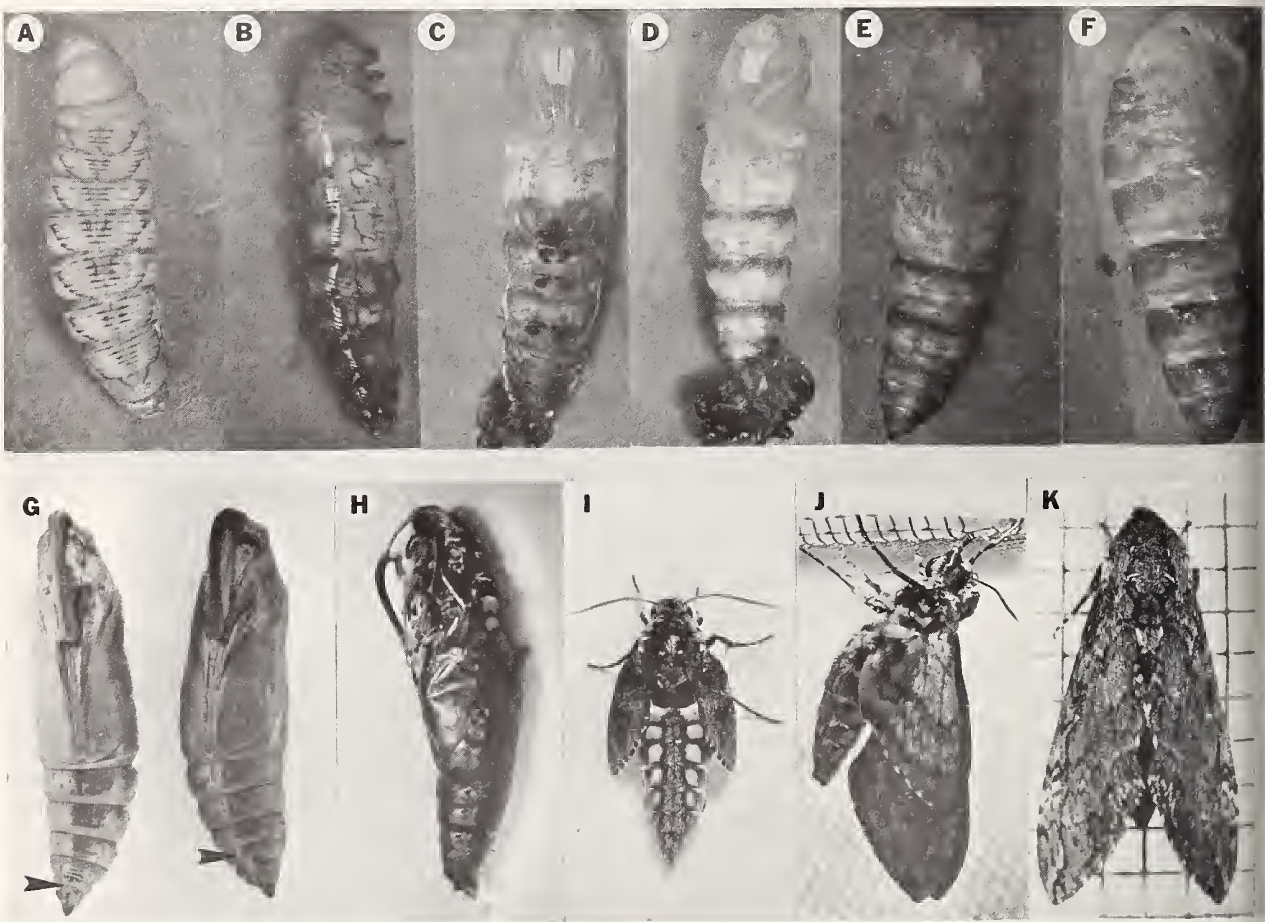

FIGURE 1.—Development of the tobacco hornworm from prepupa to adult. A, Prepupa prior to ecdysis. B, Beginning of ecdysis; white lateral strip appears when tracheal linings are withdrawn from interior (0 min). C, Ecdysis 50 percent complete (5 min). D, Ecdysis complete (10 min). E, Expansion of ventral sacs containing antennae, legs, proboscis sheath, and wings, 75 percent complete (15 min). F, Lateral view of E. G, Pupae tanned and hardened (17 h), male on left, female on right, arrows show location of sexual characters. H, Moth emerging (14 d). I, Moth expanding wings (0 min). J, Wings unfolded (20 min). K, Wings hardened (65 min).

jected that 10,000 insects could be reared weekly without modifying his procedures or equipment. Our attempts to rear 20,000 or more insects per week were thwarted by low yields resulting from contamination of the diet, despite almost daily tending of the trays, causing losses of 80 to 85 percent of the insects. Since we did not have clean room facilities available, we decided to develop a semiclosed system that not only eliminates handling of individual larvae until fully grown but also involves only two manipulations during larval feeding rather than eight or more. In addition, several diet modifications were tested to reduce costs and improve the quality of the insects produced.

PROCEDURES AND RESULTS

Oviposition

Fifty pairs of hornworms in the late pupal stage were placed in trays on the floor of a 137- by 121- by 125-cm cage for emergence. The sides and the top of the cage were burlap, a surface easily climbed by the emerging moths. While unfolding and drying their wings, the moths prefer to hang by their tarsi from a horizontal surface (fig. 1J). (See figure 1, H–K, for sequence of events before, during, and after eclosion.) Easily disturbed during this critical period, these heavy-bodied insects may be injured if they fall to the floor because of overcrowding or because of an unsatisfactory cage surface. Because they were held in a windowless room, daylight was simulated with a 60-W incandescent bulb hung from the center of the cage ceiling. The light was automatically timed to be turned on at 0600 h and off at 1800 h. A 7½-W incandescent bulb centered in an aluminum pie pan reflector remained on constantly and was adjusted with a household-type dimmer to reflect about 0.16 cd/m² of light from the feeders and oviposition substrates, which were hung from the center of the cage (fig. 2). The feeders were 16 cm and the oviposition substrates 40 cm from the top of the cage. Light outside the cage during nighttime activity must be eliminated or kept below the intensity inside the cage, since such light will override the visual stimulus inside and draw the moths away from the feeders and oviposition sites (*20*). A feeder consisted of six 10-ml vials that were friction fitted into the bottom of a 40- by 5- by 1-cm board; each vial contained a 40 percent sucrose solution. Polyethylene thistle tubes with a top diameter of 4 cm and a narrow stem that reached the bottom of the vial were used to simulate a flower. The board holding the feeder was tilted 45° from the vertical to facilitate feeding. Normally, a moth consumes an average of 0.5 ml of sugar solution per night.

Instead of tobacco leaves (*20*), we used a surrogate oviposition substrate developed by Sparks (*18*). It consists of a polyurethane foam disk (1.2 cm thick and 15 cm in diameter) sandwiched between two sheets of 0.2-cm polyethylene filter or cork (fig. 2). The upper sheet is 12.5 cm in diameter and the bottom 15 cm so that a 1.25-cm-wide band of the foam is exposed at the outer edge of the artificial leaf. The four units are attached to the holder with Velcro fasteners for easy removal and are saturated with a crude ethanol extract of tobacco, *Nicotiana tabacum* L., by using an aerosol applicator.[4] The substrate was treated at about 1600 h, 2.5 h before moth activity occurs, to avoid excessive loss of the extract but

[4] Nutritional Biochemicals Co., Cleveland, Ohio, universal kit.

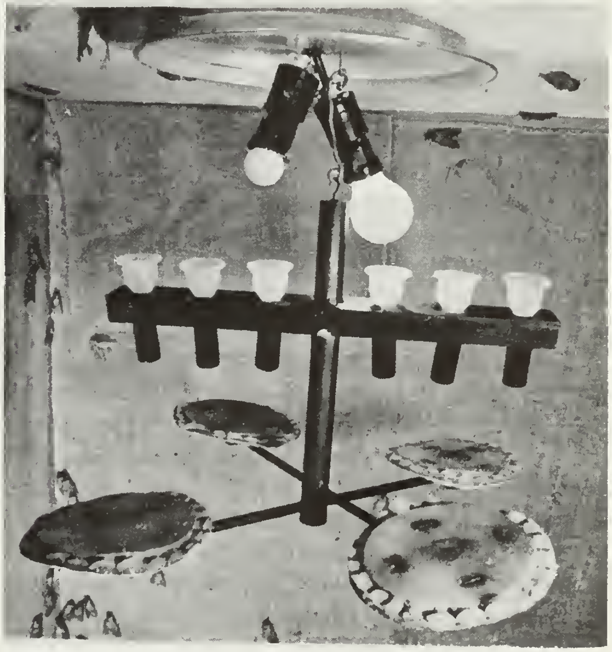

FIGURE 2.—Interior of colony cage, showing small 7½-W night light in pie pan reflector, large 60-W daytime bulb, thistle tube feeders, artificial leaves, and moths.

to allow for the alcohol to evaporate before oviposition began. The treated leaves provided the necessary visual, tactile, and chemical stimuli to induce optimum oviposition (*19, 23*). The crude tobacco extract was made by drying fresh green leaves of tobacco for 24 h at 65°–70° C. An extra large soxhlet extractor, 64-mm inside diameter, 185-mm usable depth, with a 1,000-ml flask, was loaded with about 75 g of the dried tobacco and 1,000 ml of 80 percent ethanol. When the solvent leaving the extractor was clear in color (1–2 h), the solution was bottled and stored at 5° C. Extracts containing petroleum ether and methyl alcohol that are available from chemists conducting various tobacco analyses have also been used successfully. Recently we have found that our strain of tobacco hornworms will oviposit readily on surrogate leaves treated with a solution obtained by blending 100 g of fresh tobacco per liter of water and filtering. However, the above procedure may be needed for other strains. The surrogate leaf eliminates the need for a continuous supply of fresh tobacco, since the extract can be stored indefinitely. In addition, the surrogate lasts for months and probably could be adapted to automatic collection of the eggs.

Mating of the moths begins on the first night after emergence and peaks the second night. Egg production begins the third night, peaks the eighth, and dwindles rapidly thereafter. About 85 percent of the eggs are produced by the 12th day. Although potential egg production is almost 1,400 eggs per female (*20*), caged females averaged 700– 800 eggs during an average lifetime of 15 days, somewhat more eggs than the 500 recorded by Yamamoto (*20*). The relative humidity is held at 75–85 percent and temperature at 27°–30° C. Low humidity before emergence desiccates the moths and causes a high rate of wing deformity. After emergence, mating and oviposition are greatly suppressed by low humidity. Excessive humidity (saturation) is also undesirable, since it will completely suppress activity.

Egg Preparation

In the field, the tobacco hornworm lays its eggs singly. In the laboratory, however, these reared hornworms, given the crowded conditions, lay eggs concentrated in a band on the bottom edge of the artificial leaf. Maximum oviposition expected per night is 1,250 eggs per leaf, or 5,000 per cage. The relatively large, durable hornworm eggs (fig. 3) are easily removed with the thumb from the artificial leaves. Before being placed in starting containers, the eggs are surface sterilized for 15 min with a 5.25 percent sodium hypochlorite solution (Chlorox) diluted 1:200 with tapwater, rinsed in tapwater for several seconds, and dried promptly in a clean-air hood. The sterilizing solution, in addition to cleaning the egg surface, also aids in separating the eggs, which tend to come off in large clusters. This separation is essential for accurate measurements of the eggs, which can be weighed (about 750 eggs/g) or, because of their large size, measured volumetrically with a small scoop (about 450 eggs/cm^3). For accuracy, the eggs must be weighed at a uniform age, since weight is reduced 20 percent from the time of collection to full development because of the metabolism of the developing embryos.

Diet Preparation

In view of the success of Burton (*3*) in rearing the corn earworm, *Heliothis zea* (Boddie), on a corn meal, soy flour, nonfat dried milk, and soy oil combination (CSM), we replaced the wheat germ, sucrose, and salt mixture (table 1) in the Yamamoto (*21*) diet with CSM. However, CSM, which was developed as

FIGURE 3.—Closeup of hornworm eggs being removed from surrogate leaf.

a complete diet for human consumption in protein deficient countries, apparently does not have the proper balance of amino acids for the tobacco hornworm. Casein or yeast or both are needed as a supplement, though the amounts can be varied according to cost and availability, as long as the protein level does not drop below 35 percent dry weight. Gelcarin was substituted for the commonly used agar because of its lower cost, the smaller quantity needed, and the lower temperature required for mixing. If CSM is not available, a mixture of 65 percent yellow corn meal and 35 percent toasted soy flour can be substituted. The other ingredients are not required except for the mineral premix, which can be supplied by adding 0.6 g NaCl and 0.6 g $CaCO_3$ per liter of water.

The carotenoids present in the corn meal plus the vitamin A present in the CSM and vitamin premix produce larvae more normal in color, that is, light green versus the teal-blue color of larvae reared on the wheat germ and casein-yeast diet of Yamamoto (*21*), who, however, was able to correct this color partially by adding tobacco. Larvae reared on natural host plants are normally a medium green. The importance of vitamin A in the diet was further demonstrated by Carlson et al. (*6*), who found pathological changes in the ultrastructure of photoreceptor cells in hornworms reared on a diet deficient in vitamin A. These findings corroborated the work of Sparks (*17*), who found that hornworms reared on a diet lacking vitamin A responded similarly in the laboratory to those reared on tobacco but required a higher light level to induce oviposition and mating activity.

The diet was mixed in a large 160-l soup kettle (fig. 4) equipped with a 5,000-W submersible heater. First, 90 percent of the water was heated to 77° C. All the ingredients except the formalin were combined and poured into the kettle as the water was agitated vigorously with a commercial clamp-on mixer equipped with two 9-cm-diameter propellers and powered with a one-third-hp direct-drive (1,750-r/

TABLE 1.—*Ingredients of hornworm diet*

[Makes 1.25 liters][1]

Ingredient	Amount ($g/l\ H_2O$)[2]
NUTRIENTS	
CSM[3]	120
Casein (sodium caseinate)	16
Torula yeast	64
Cholesterol	1
Vitamin premix[4]	10
MICROBIOCIDES	
Sorbic acid	2
Methyl-*p*-hydroxy benzoate	1
Formalin (40% formaldehyde)	4.7
Streptomycin sulfate	.2
GELLING AGENT	
Carageenan HWG (gelcarin)	13

[1] Cost: $0.38 per 1.25 l at 1977 prices.

[2] Amount of formalin in $ml/l\ H_2O$.

[3] 63.8% yellow corn meal gelatinized; 24.2% soy flour, toasted; 5.0% nonfat dried milk; 5.0% soy oil, refined; 1.9% mineral premix; and 0.1% vitamin premix. Companies manufacturing CSM include: Krause Milling Co., 4222 W. Burnham St., Milwaukee, Wis. 53246; Lauhoff Grain Co., Danville, Ill. 61832; Archer Daniels Midland Co., 4666 Faries Parkway, Decatur, Ill. 62521.

[4] Prepared by Hoffman-LaRoche, Inc., Nutley, N.J., as specified by the USDA Biological Control of Insects Research Laboratory, Columbia, Mo. Contains 50% ascorbic acid and 12 additional vitamins in a dextrose base.

FIGURE 4.—Diet being drawn from soup kettle into finishing tray.

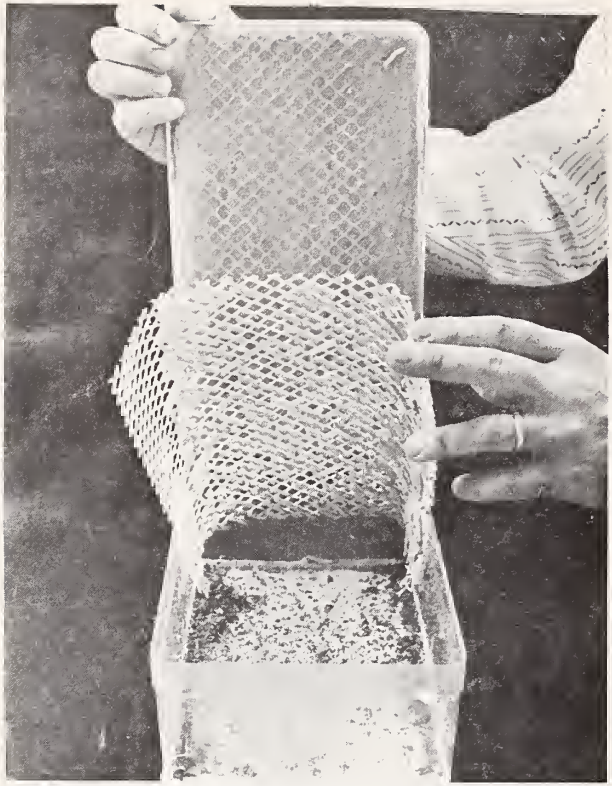

FIGURE 5.—Starting container with 5-day-old larvae.

min) electric motor. As soon as the diet was well mixed (2 min), the formalin and the remaining 10 percent of the water (unheated) were added. The unheated water drops the temperature to 68° C and prevents overheating that may alter any heat-labile materials. In addition, the lower temperature increases viscosity, thus preventing settling of the ingredients before gelling, which occurs at 57° C. Mixing was continued for 5 min. The diet was dispensed into rearing containers: the lids of the starting containers were filled to a depth of 1.3 cm (0.6 l), and the finishing trays were filled to a depth of 3.7 cm (10 l). The diet was covered with sheets of wrapping paper to exclude contaminants and stored in a cool room until used, usually in 1–8 days. However, if the filled starting container lids are to be stored for an extended period after cooling, they must be refrigerated at high humidity, preferably in sterile plastic bags, to prevent drying and separation of the diet from the edges of the container.

Starting Containers

The starting containers were made from commercially available transparent plastic shoe boxes 32 by 17 by 9 cm (fig. 5). The bottom was cut out, leaving a 3-cm interior lip to which was glued a 70-μm polyethylene filter 0.15 cm thick that allowed adequate air moisture exchange. About 350 eggs were placed loosely on the polyethylene filter, which must be kept dry to avoid a poor hatch. A 1-cm mesh, 60 CDS 39 Vexar plastic screen 26 by 30 cm was folded in the form of an inverted U and placed in the box to support the diet and to provide a perch for the developing larvae. The lid containing the diet was placed on the box and secured by two rubber bands. (The diet provides an adequate seal to prevent escape of the newly hatched larvae.)

As Yamamoto (*21*) showed, newly hatched hornworm larvae are strongly photopositive. Lighting must be uniform to prevent congregation before establishment on the diet, or high

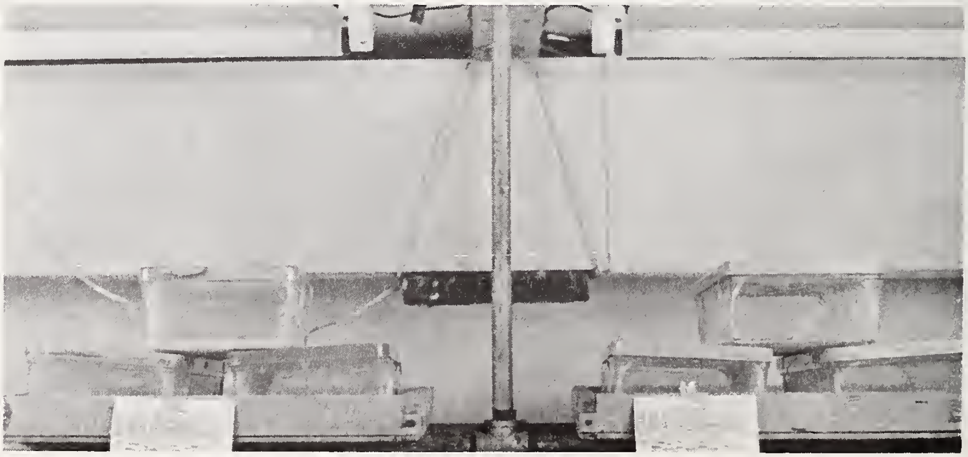

FIGURE 6.—Starting containers in screen trays under single flourescent tubes.

losses will occur because of fighting, nipping, and the tendency of one larva to attach to the horn of another. Adequate lighting can be achieved by centering the containers under single fluorescent tubes (fig. 6). For convenience, constant light is provided, but it could be turned off after 2 days, when the larvae have become established. Condensation must be avoided since the tiny larvae are easily trapped in water droplets. Also, later in the developmental period, high moisture will interfere with drying of the fecal pellets and foster bacterial and fungal growth. Therefore, the relative humidity in the holding room is kept at 55–60 percent and the temperature 26°–30° C. However, the vertical humidity gradient in the container prevents excessive drying of the diet at the top and at the same time permits drying of the fecal pellets at the bottom. The container must be held in screen trays or spaced on open shelving to permit ventilation underneath. The starting containers contain sufficient diet and space to accommodate about 300 larvae for 5 days, at which time most have reached the third instar.

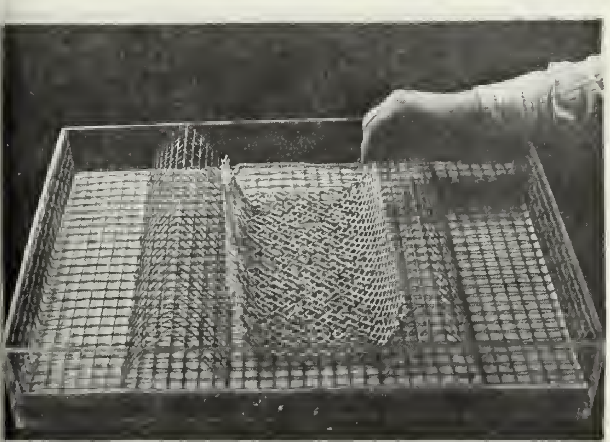

Figure 7.—Support screen from starting container with 5-day-old larvae being transferred to finishing tray (inverted).

Figure 8.—Finishing tray complete with fine-mesh Vexar screen and T-bar.

Finishing Trays

The finishing trays are 62 by 44 by 11 cm and made of Plexiglas 0.6 cm thick. A 1.6-cm-mesh hardware-cloth unit is inserted to support the diet and provide a perch for the older larvae. When it is time to move the larvae from the starting container to the finishing tray, the Vexar screen from the starting container with the 5-day-old larvae clinging to it is placed between the two V-shaped extensions of the hardware cloth support (fig. 7). A 0.3-cm-mesh 20 PDS 129 Vexar screen is positioned over the

Figure 9.—Finishing rack (0.5 by 1.2 by 1.9 m) with 16 trays and collection units (produces 4,800 prepupae weekly).

tray and held in place with a frame made from a 5-cm-wide T-bar with a 1.6-cm projection (fig. 8). The screen prevents premature crawloff by the larvae and provides aeration to dry the fecal pellets, and the T-bar provides rigidity. The assembled tray is inverted and placed on an open rack (fig. 9) and held in the same room as the starting trays for the 3 days required for the larvae to develop to the fifth stadium. The larvae feed from underneath (fig. 10), so that as diet is consumed, the remaining diet moves downward to stay in contact with the support screen. When most of the diet is consumed, some larvae will feed from the top or side of the diet (fig. 11).

During the fourth stadium, the larvae consume about 0.5 l of diet, but during the fifth and final stadium, which lasts about 3 days, the larvae consume 90 percent of their total diet, about 30 ml each, 9 l for 300 larvae. During this period, fecal pellets may accumulate 5–7 cm deep if they are not dried. Adequate drying is assured by transferring the finishing trays to a room where fans are used to move the air beneath the trays at a velocity of about 30 m/min. Temperature and humidity in the room are similar to those in the starting area, RH 55–60 percent and temperature 26°–30° C.

After 3 days in the fifth stadium, prepupae begin seeking a place to pupate. A flap is opened at one end of the 0.3-cm-mesh Vexar-screen bottom so that the prepupae can exit and drop into a collection tray beneath (fig. 12). Since 80 percent of the prepupae leave the finishing tray within 6 h after lights are off, we keep the insects from long periods of wandering about in the collection trays by timing the lights to turn off at 2300 h and on at 0800 h, when they are collected.

Before tobacco hornworms seek a place to

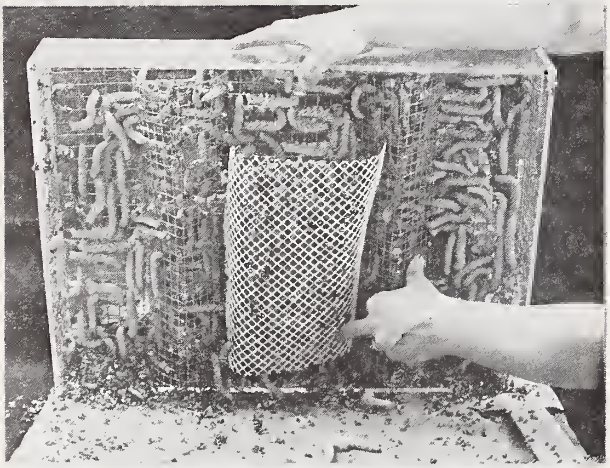

FIGURE 10.—Finishing tray raised to show large 11-day-old fifth-instar larvae feeding underneath.

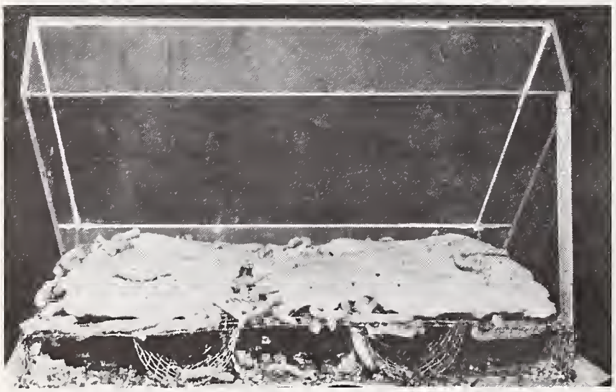

FIGURE 11.—Top view of finishing tray.

FIGURE 12.—Flap opened to allow prepupae to drop into collection tray (one rearing tray removed to expose flap).

pupate, they spend about 6 h resting and grooming themselves. Starting at the anterior, they slowly move their mouthparts in an arc dorsoventrally over almost the entire body several times, first one side then the other, covering it with a fluid. The purpose of the grooming process is unknown.

Pupation

The prepupae are taken from the collection trays and placed in units containing 160 individual wooden cells each 2.5 by 2.5 by 10 cm (*10*) (fig. 13). A 0.6-cm-mesh hardware cloth is stapled to one side of the unit to allow ventilation, and the other side is covered with a sheet of 1-cm-thick marine plywood held in place with two rubber bands cut from a large truck inner tube. These units are kept in a holding room at 75–80 percent RH and 26°–30° C. The units are positioned so that the prepupae are resting horizontally. The surface on which the prepupae rest must be free of sharp projections to avoid punctures in the delicate pupal cuticle before it hardens. Unless the room is darkened, the prepupae may attempt to escape or go through the motions of building a cell, or both, for several days. However, we have not found any detrimental effects from this activity. At 4 days, the prepupae shed the final larval skin and molt to the pupal stage (fig. 14).

The pupal stage is a critical time in the insect's life. If excessive desiccation has occurred because of low humidity or excessive activity because of a delay in placement in the pupation cells, the insect may be unable to pump enough blood into the anterior region to break the seam in the larval skin. The seam may be broken, but a lack of lubricant between the larval skin and the pupa may prevent complete ecdysis. If the prepupa is lying on a wet surface, the larval skin will become pliable and stretch rather than break at the seam. If molting is successful, the insect still faces the critical pumping of blood into the ventral sacs to expand them into their normal position. These sacs contain the developing wings, antennae, legs, and proboscis of the adult. If the pupa is tilted to one side, the weight of the sacs will cause them to slide off course as they move posteriorly, exposing the tender area normally covered by the sacs. Since this area is incapable of hardening or tanning, the insect soon dies from desiccation or loss of blood. If the insect is lying in the proper position but the blood volume is low, tender areas will again be left exposed because the insect is incapable of expanding the various sacs to their normal position. Even if the insect survives, the adult will be deformed because of improper expansion and knitting of the various sacs. Various stages in molting and expansion of the pupal sacs are shown in figure 1, A–F.

At least 24 h are required for the pupae to tan and harden (fig. 1G). To insure that all the pupae are hardened, we do not harvest them until 7 days or more after the prepupae are placed in the cells. When pupae are to be collected, the rubber bands are removed, and the pupation unit is placed gently on a table with the wooden cover down. In this way, the pupae can slide out of the unit as it is lifted from the cover (fig. 15). The pupae are transferred to screen trays in single layers and stored at the same conditions as in the pupation area.

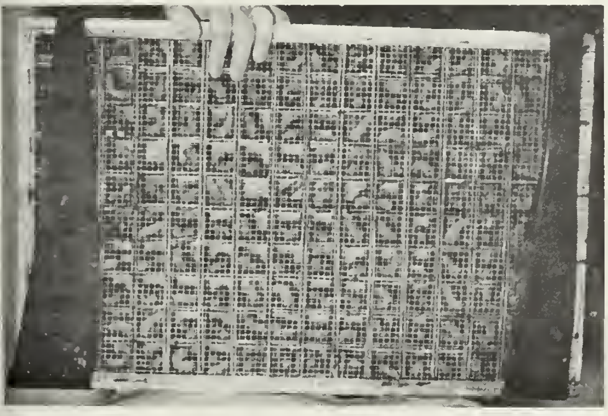

FIGURE 13.—Prepupae in unit containing 160 cells.

FIGURE 14.—Pupae in cells ready for collection (7 days after placement of prepupae).

TABLE 2.—*Equipment, material, and manpower to rear about 20,000 tobacco hornworms weekly*

Operation	Setup: Units	Setup: Items per unit	Total yield	Man-hours	Days elapsed
Set up colony cage	1	100 pupae	...	1	...
Collect, disinfect, and dry eggs	14	2,500 eggs	35,000	1	...
Weigh diet ingredients and mix	4.6	160 liters	735 liters		
Draw diet into starting containers	70	0.5 liters	35 liters	[1]6	...
Draw diet into finishing trays	70	10 liters	700 liters		
Set up starting containers	70	350 eggs	[2]24,500	1.5	0
Transfer 5-day-old larvae	70	310 larvae	21,700	2.2	5
Place prepupae in cells	136	160 prepupae	21,700	7	11–13
Collect pupae	136	158 pupae	21,000	4.7	20
Rearing total				23.4	
Clean and sterilize equipment				6.5	
Clean and disinfect working areas				5	
Cleanup total				11.5	
Total				34.9	

[1] Tasks combined for maximum efficiency, for example diet ingredients can be weighed during the 30 min required to heat water.

[2] 10,000 surplus eggs not used.

FIGURE 15.—Pupae on cover of pupation unit.

In an alternate procedure, prepupae are placed in a soil substitute for pupation. A tray 50 by 38 by 9 cm deep will accommodate 50 pupae. A 1:1 mixture of fine, firmly packed vermiculite and sand dampened with tapwater to the point that it still flows (readily sifts through a 0.6-cm-mesh screen) has given yields equal to those obtained with the wooden cells. However, if the soil mixture contains coarse material, the prepupae will either spend considerable time attempting to chew the material into small particles or will reject the site. Also, the pupation medium must be at least 8 cm deep to allow for construction of a durable cell. And placement of prepupae in a tray must be synchronous; otherwise late arrivals will burrow through the walls of cells constructed previously by early arrivals. The trays must be held at 85 percent RH or loosely covered to prevent excessive drying. After the pupae are thoroughly hardened, they may be gently sifted from the medium. Disadvantages of this method are increased space and weight, the need to sift pupae, and the need either to replace or sterilize the medium. However, for small-scale or intermittent production it may be the best method, since the need to construct wooden cells would be avoided.

In either method, the pupation site must be absorbent to prevent the prepupa from drowning in the large amounts of fluid produced as it grasps soil particles in its mouth to "plaster" the actual cell wall (or merely goes through the motion when a wooden cell is provided).

Sanitation

All rearing equipment is routinely soaked overnight or longer in a 0.5 percent Chlorox solution in large vats for cleaning and sterilization. Equipment in storage is soaked again just before use. The same solution is used to mop floors, walls, and working surfaces. Any units that contain unhealthy larvae or that have active colonies of bacteria or fungi growing on the diet or fecal pellets are removed from the rearing area as soon as observed. In addition, the pupation units are held 2–3 s in a 1.5 g/l

solution of streptomycin sulfate to provide a residue of antibiotic, since the chlorine rapidly dissipates.

Production Efficiency

With the described procedures, we have maintained an 85 percent recovery from egg to normal adult. Losses normally occur as follows:

Mortality factors	*Percent loss*
Nonviable eggs	5
Failure to establish in starting trays	6
Failure to pupate	3
Failure to emerge as sound adults	1
Total	15

Although only 0.5 percent of our production is needed to maintain our colony, 1.5 percent is held routinely to assure adequate egg production. One man working part time at the Oxford laboratory has consistently produced 6,000–7,000 pupae per week, and we estimate that on a full-time basis 20,000 could be produced (table 2). Two-thirds of the time spent (23.4 h) is used in rearing the insects and one-third (11.5 h) in cleanup. On St. Croix, two men used the technique to produce 30,000–35,000 pupae per week for sterile-male releases (*16*). The rearing of as many as 200 million per year for sterile releases as proposed by Knipling (*13*) would justify assembly-line procedures as used for the screwworm, *Cochliomyia hominivorax* (Coquerel) (*2*). Such a streamlined procedure would reduce the man-hours used an estimated 44 percent.

Adaptation of Methods to Other Insects

During the first 2 years our technique was used with the tomato hornworm, *M. quinquemaculata* (Haworth), this insect, colonized in August 1973, did not respond well. Mating and egg hatch were low, and tobacco or tomato foliage had to be used for oviposition instead of the surrogate leaf. According to Yamamoto (*22*), the tomato hornworm prefers to fly at a lower elevation than the tobacco hornworm. We therefore lowered the feeders from 16 cm from the top of the cage, as used for the tobacco hornworm, to 64 cm. We also lowered the two sets of tobacco leaves from 40 to 64 cm (tip of leaves to top of cage) and from 60 to 84 cm, respectively. Immediately, average consumption of sucrose per moth increased from 2.10 ml to 4.04 ml, egg production per female increased from 303 to 631, and the percentage of virgin females decreased from 17.4 to 12.2 percent. When we retested the surrogate leaf in December 1975, oviposition was greatly improved and averaged about 300 eggs per female. The only other difficulty with the system is the tendency of about 10 percent of the culture to remain in the rearing tray after feeding is complete, meaning that the prepupae must be hand collected.

The system now shows some promise for rearing a beneficial sphingid, *Hyles euphorbiae* (L.), that is to be released for the biological control of leafy spurge, *Euphorbia esula* L. (P. H. Dunn, 1974, personal communication).[5] The system has had only limited success with the cabbage looper, *Trichoplusia ni* (Hübner). Up to 1,000 larvae per finishing container grew well, but large losses occurred during pupation. However, additional space for pupation or reduced lighting may be required. Normally, the cabbage looper is reared in small opaque containers (*9*). With the tobacco budworm, *Heliothis virescens* (Fabr.), the starting container has worked fairly well. Yields 30–40 percent, that is, 90–120 pupae, are obtained from 300 eggs. (The tobacco budworm prepupae are not removed but are allowed to pupate in the bottom of the container under four sheets of paper toweling.)

Shipping Pupae

After testing vermiculite and several other packing materials, we found that sandwiching tobacco hornworm pupae in a 12-layer absorbent paper cushion gave the best results. The paper cushion is cut into 30- by 90-cm sections, and the top six layers are removed. Pupae weighed out into 450-g lots (to yield about 100 pupae each) are evenly spaced in 3 rows parallel to the long edge of the cushion. The top six layers of cushion are replaced, and the entire unit is rolled up along the long axis [parallel to the short edge (fig. 16) to avoid crushing the pupae] just tight enough to prevent shifting of the pupae. The completed roll is secured with rubber bands at both ends (fig. 17). A

[5] Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif. 94706.

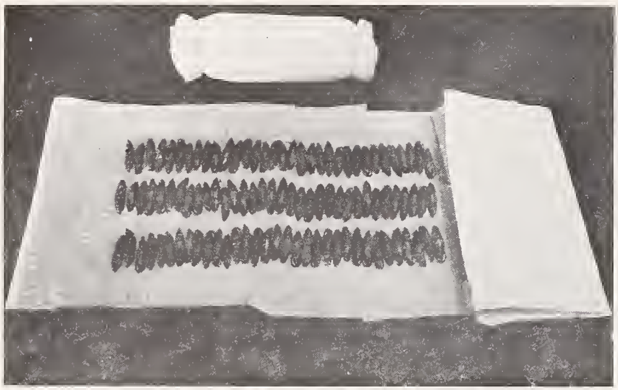

FIGURE 16.—Pupae (100) placed on bottom 6 layers of paper cushion before being covered with top 6 layers.

30- by 30- by 30-cm carton will hold eight rolls. Hundreds of shipments have been made with only 2–3 percent loss in route, unless there were long delays or exposure to lethal temperatures.

Vermiculite and other loose packing materials allowed the pupae to concentrate at the bottom of the container. The result was overheating, broken proboscis sheaths, and body punctures from the sharp abdominal cremasters at the posterior of the squirming pupae. Attempts to use flat layers of cushioning material were thwarted by the difficulty of holding the pupae in place without crushing them.

DISCUSSION

For many years, the tobacco hornworm has been a serious insect pest of tobacco. As a result, Knipling (*13*) proposed the integration of early stalk destruction (*15*) and the sterile male technique to eliminate the tobacco hornworm from 1,250,000 acres (including about 400,000 acres of tomatoes) east of the Mississippi River. We now have an acceptable method of mass rearing sufficient numbers of the insect for such releases and have demonstrated the feasibility of using sterile males to suppress the native population on St. Croix, U.S. Virgin Islands. The next step would involve testing the concept on a larger scale in the continental United States.

Although the sexing of tobacco hornworm pupae is time consuming if done by hand, this operation undoubtedly could be automated because of the size of the insect and the readily distinguishable sex characters (fig. 1G). This would facilitate release of sterile females in areas separate from sterile-male releases or the utilization of fertile females to produce hornworm eggs for mass rearing and release of an egg predator, the spined stilt bug, on tobacco (*7*), and *Trichogramma* sp. and *Telenomus* sp. as egg parasites on crops other than tobacco. Larvae could be used to rear *Apanteles congregatus* (Say) or tachinid spp. to fill the early-season void in parasites that normally exists. Other uses involve synthesis of sex pheromones and studies of population density and movement.

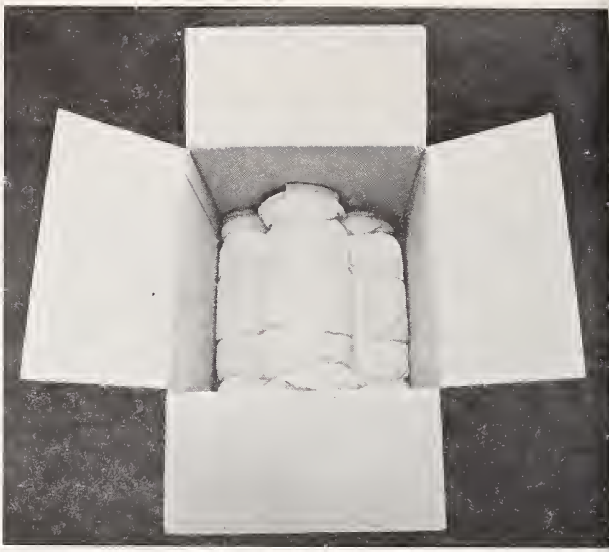

FIGURE 17.—Cushions containing pupae, rolled and secured with rubber bands, ready for shipping.

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